ABSENCE OF KIN DISCRIMINATION BEHAVIOR IN A SOLDIER-PRODUCING APHID, *CERATOVACUNA JAPONICA* (HEMIPTERA: PEMPHIGIDAE; CERATAPHIDINI)

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Abstract.—Certain aphid species produce sterile soldiers, morphologically and behaviorally specialized individuals that defend fertile colony members, some or all of which are clonemates. If the soldier morph is maintained by inclusive fitness advantages, its altruism should preferentially benefit relatives, suggesting a potential role for kin discrimination. We performed a field experiment on spatial segregation and two laboratory experiments on agonistic behavior among non-soldiers and by soldiers of the cerataphidine aphid, *Ceratovacuna japonica*. For the test of spatial segregation, we introduce a new method of nearest-neighbor analysis, by constructing a minimum spanning tree from the map of individual locations and comparing the numbers of within-group and between-group connections. The results provide no evidence of kin recognition abilities in this species. Members of different clones showed no tendency to segregate spatially, nor to direct displacement attempts against non-kin when competing for feeding sites. Soldiers were indiscriminately aggressive toward early instar reproductives from their own and other colonies. We discuss the implications of these findings for several evolutionary hypotheses on the maintenance of the soldier morph in aphids.

The discovery of sterile aphid soldiers (Aoki, 1977) has been widely interpreted as confirming a prediction of inclusive fitness theory, that altruism can evolve within clones of cyclically parthenogenetic organisms (Dawkins, 1979; Aoki, 1987; Hamilton, 1987; Ito, 1989). Soldiers are first or second instar individuals which are morphologically and behaviorally specialized for colony defense and which, in failing to molt to adulthood, do not reproduce. Soldier morphs have evolved independently at least four times among pemphigid aphids, and soldier-like behavior by monomorphic first instars, which do become reproductive adults, also occurs in some species (Aoki and Kurosu, 1987; Kurosu and Aoki, 1988; Moran, 1993). Attacks by soldiers and monomorphic defenders on vertebrates and the eggs and larvae of predatory insects have been documented in a number of studies (Aoki, 1979; Ohara, 1985; Aoki and Kurosu, 1987; Kurosu and Aoki, 1988; Foster, 1990). If the colony originated from one parthenogenetic foundress, soldiers protecting fertile colony members would promote the reproduction of their clone, a role analogous to that of somatic cells in a metazoan body (Dawkins, 1979; Hamilton, 1987).

However, were some females to join a colony but produce fewer or no soldiers, the "cheaters" could enjoy enhanced reproductive success at the expense of the soldier-producers (Aoki, 1980; Hamilton, 1987). The proportion of soldiers is highly labile both between colonies and, over time, within colonies (Sunose et al., 1991;

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Sakata et al., 1991), suggesting that there may be some variation in soldier-producing propensity, which could facilitate the generation of cheating mutants. Ito (1989), summarizing suggestions of previous authors (e.g. Aoki, 1987; Hamilton, 1987), offered three hypotheses for the evolution and maintenance of soldier production under these circumstances: (1) soldiers recognize and expel unrelated genotypes, preserving clonal integrity; (2) dispersing females join established colonies so infrequently that genetic mixing is unlikely; or (3) predation pressure requires the maintenance of a certain proportion of soldiers even if colonies are genetically diverse, while failure to produce soldiers decreases colony fitness. The experiments reported here were designed to test an expanded version of the kin recognition hypothesis. Kin discrimination by soldiers, and/or by reproductives, could preserve effective clonal integrity by the exclusion of most or all foreign intruders, as in Ito's hypothesis 1 (henceforth referred to as hypothesis 1a). Alternatively, discrimination could enable soldiers preferentially to defend clonemates within a genetically diverse colony (hypothesis 1b). E.g., if the usually sedentary reproductives form spatially segregated, clonal subgroups, kin recognition could enable the mobile soldiers to remain in the vicinity of their clonemates.

In addition, apparently agonistic interactions within colonies have been observed in the form of "butting" behavior, by which aphids attempt to displace fellow colony members from feeding sites (Aoki et al., 1981; Aoki and Kurosu, 1985). Aoki (1987) reported intracolonial butting in all species studied in the tribe Cerataphidini, including soldier-producing and monomorphic species, and speculated that the defensive behavior and frontal horns of soldiers derived from butting by monomorphic precursors. Noting that conflict is unexpected within pure clones, Aoki suggested that such colonies might indeed be genetically diverse, in contradiction to hypotheses (1a) and (2), but consistent with (1b) or (3). (Other interpretations of butting are possible; see Discussion.) Unfortunately, no data on the genetic composition of colonies are yet available for any soldier-producing aphid species. However, the hypothesis that kin discrimination is used to direct butting toward non-clonemates can be tested.

Ceratovacuna japonica (Hemiptera: Pemphigidae; Cerataphidini) produces "pseudoscorpion-like" first instar soldiers, with prolonged, sharp frontal horns and thickened, grasping forelegs (Aoki, 1977, 1987). Dense clusters of these woolly aphids are commonly found on the underside of bamboo grass leaves (*Pleioblastus* spp.), the secondary host (Aoki and Kurosu, 1991). For the purpose of this study, a "colony" is functionally defined as an aggregation of aphids on one leaf, which can benefit from defense by the soldiers present on that leaf. The inhabitants of multiple leaves on the same or neighboring plants may constitute one genetic population, however. Production of an alate sexual phase, migration to a primary host and gall formation are uncommon in this species; in fact, its gall generation and primary host (*Styrax japonica*) have been discovered only recently (Aoki and Kurosu, 1991). Most colonies are founded asexually by non-soldier first instars, dispersing on the wind to new plants (Pierce and Berry, unpubl.). In dense populations, over the lifetimes of colonies, it seems possible that a dispersing female may arrive on an already occupied host plant, but the frequency of this event remains unknown.

We tested *C. japonica* aphids for recognition of colonymates and putative clonemates in the contexts of spatial segregation, butting and soldier aggression. Incipient colonies separated by large distances were used in the first two experiments because

they were more likely to be pure clones, still comprised only of offspring of the first female to arrive on the plant. Since incipient colonies contain few soldiers, larger colonies provided soldiers for the third experiment, though an unknown number of subsequently arriving females may have contributed to these colonies. Were these aphids capable of recognizing kin as required by hypotheses (1a) or (1b), we expected to observe discrimination against members of foreign colonies in at least one of these three behavioral contexts.

METHODS

Experiment 1—Spatial segregation. Experiments were conducted in the laboratory of Dr. S. Aoki and in stands of bamboo grass (*Pleioblastus chino*) in the vicinity of Rissho University, Kumagaya, Saitama Prefecture, Japan, in July 1988. In all experiments, *C. japonica* colonies assumed to be unrelated were collected from sites at least 1 km apart. For experiment 1, the test of spatial segregation, 56 incipient colonies were collected, containing either one adult aphid with a group of early instars (22 ± 11 each, mean ± SD, N = 16 colonies), or a group of early instars without an adult (20 ± 12 each, N = 40). Incipient colonies containing one adult female were presumably all offspring of that adult. Of those without an adult, most were probably the offspring of one recently-deceased foundress, since it seems unlikely that large numbers of independently wind-dispersed individuals would arrive synchronously on one leaf. The results obtained with both types of colony did not differ (see Tables 1, 2). Most incipient colonies do not contain soldiers; two of this sample of 56 included one soldier each, and one included two soldiers.

Pairs of colonies from different sites, containing approximately equal numbers of individuals, were marked with different colors of fluorescent dust (Crumpacker, 1974), which is readily absorbed by the white waxy secretions that coat these woolly aphids. Individuals were placed one by one, alternating the colony of origin, onto a marked spot on the upper surface of an unoccupied bamboo grass leaf in the field. The aphids quickly moved to the underside of the leaf, allowing the placement of the next individual on the same spot. After 24 hours, the leaf was cut and the positions of all marked individuals remaining on its lower surface were recorded.

To test for non-randomness in the distributions of the two groups, we employed a new method of nearest neighbor analysis, based on the construction of a minimum spanning tree between observed data points. Pielou's (1961) contingency table procedure has been the standard method for analysis of nearest neighbor frequencies. However, Meagher and Burdick (1980) demonstrated that nearest neighbor pairs are often not independent, violating the assumption of the χ^2 test. If individual A is B's nearest neighbor, B is likely to be A's, so some pairs are counted twice. Meagher and Burdick proposed an alternative significance test, which requires extensive computer simulation of randomized data sets, but stated that a simpler test would be preferable. We introduce here a simple test of spatial association that can be carried out manually.

Given a map of the locations of N individuals belonging to groups 1 through k, N-1 point-to-point connections can be drawn so that there is a path between any two points, and the summed length of all connections is minimized. This construction, a minimum spanning tree, contains all nearest neighbor pairs if the interpoint distances are unique (Cheriton and Tarjan, 1976). The connections between points are independent, in that reciprocal pairs cannot occur. The procedure for constructing a

Table 1. Spatial aggregation in pairs of *C. japonica* colonies, by minimum spanning tree method for nearest-neighbor analysis, described in the text. Observed and expected numbers of nearest-neighbor connections between members of the same colony are compared by χ^2 test; n.s. = not significant at $P > 0.05$. Colonies are numbered arbitrarily; those marked with an asterisk contained an adult.

Replicate	# Individuals		# Same-colony neighbors		Significance
	Col. 1	Col. 2	Obs.	Exp.	
1	11	5*	8	8.1	n.s.
2	20*	7	15	15.6	n.s.
3	7	17	15	13.1	n.s.
4	20*	10*	18	15.7	n.s.
5	7	17	12	13.1	n.s.
6	6	8	5	6.1	n.s.
7	5	9	5	6.6	n.s.
8	14	13	13	12.5	n.s.
9	22*	19	24	19.6	n.s.
10	16*	29*	26	23.4	n.s.
11	9	4	6	6.5	n.s.
12	34	31	33	31.6	n.s.
13	6	9	10	6.8	n.s.
14	6	28	24	23.1	n.s.
15	5*	21	21	16.9	n.s.
16	3	24	22	20.7	n.s.
17	12	24	18	19.0	n.s.
18	10*	15*	12	12.0	n.s.

minimum spanning tree for a given data set is as follows: (1) Pick any point to be first in the tree; (2) Find the point not yet in the tree which is closest to any point in the tree, and connect it to the latter; (3) Repeat step 2 until all points are included (Even, 1979). The frequencies of within-group and between-group connections can be arranged in a contingency table. Since the order of the connected points is unimportant, we adopt the convention that the table be lower-diagonal; i.e., the observed frequency $f_{i,j}$ is the number of (i,j) and (j,i) connections. Letting n_i denote the number of members of group i, the expected frequencies are $e_{i,j} = n_i(n_i - 1)/N$ for $i = j$; and $e_{i,j} = 2n_i n_j/N$ for $i > j$. For the χ^2 test, the table has $k(k - 1)/2$ degrees of freedom. This method has been tested by Monte Carlo simulation for sample sizes between 20 and 100, and performs as expected (Gladstein, unpubl.).

Experiment 2—Butting behavior. To test for discrimination in butting, 20 incipient colonies were collected. Nine of the colonies included one adult and 16 ± 8 early instars each, 11 colonies without an adult contained 17 ± 10 early instars each, and 10 also included one or two soldiers. (Incipient colonies with soldiers were selected for this experiment, since potential competition for feeding sites by non-reproductives is of particular interest.) Sections of leaves on which the aphids were feeding were excised and taken to the laboratory, where pairs of colonies from different sites were color-marked with fluorescent dust and their leaf sections fixed upside down in a petri dish, with the margins of the two groups abutting. After several hours, as the

Table 2. Agonistic interactions (butting) between pairs of *C. japonica* colonies. Observed and expected numbers of interactions between individuals from different colonies are compared by χ^2 test; n.s. = not significant at $P > 0.05$. In replicates marked with an asterisk, one colony contained an adult; in those marked with two asterisks, each contained an adult.

Replicate	Total # interactions	# Betw.-colony interactions		Significance
		Obs.	Exp.	
1	121	49	64	$P < 0.005$
2*	133	58	58	n.s.
3*	52	24	23	n.s.
4*	86	50	45	n.s.
5*	106	47	50	n.s.
6	202	96	90	n.s.
7**	70	23	28	n.s.
8*	170	63	62	n.s.
9	24	11	11	n.s.
10**	53	24	27	n.s.

cut leaves began to dry, individual aphids from both colonies ceased feeding and began to wander among those still feeding.

Butting behavior, in which a mobile individual thrusts its head against a feeding individual in an apparent attempt to displace it from the feeding site (described by Aoki and Kurosu, 1985), was recorded in a series of 1 hour sessions, totalling 19 hours. Most colony pairs were observed for 2 hours, but pairs 2 and 9 were observed for only 1 hour, after which no aphids were feeding and butting ceased; and pair 1 was observed for 3 hours. We recorded the colony of origin, morph and size (adult or early instar; no late instar juveniles were present) of the interactants, and success or failure in displacing the victim from its feeding site. Single butts were uncommon; most interactions consisted of several or many separate thrusts. Repeated thrusts against the same individual were scored as one interaction unless the attacker walked off, then returned to butt its previous victim again. To test the null hypothesis that butting is independent of colony membership, the observed scores of interactions between and within groups were arranged in a contingency table, and the values expected for each cell were obtained by cross-multiplying the marginal totals:

$$\text{Expected [i butts j]} = \frac{\text{observed [i butts]} \times \text{observed [j is butted]}}{n},$$

where n is the total number of interactions per colony pair. Expected and observed values in the 2 × 2 tables were compared by χ_1^2 test. For brevity of reporting, only the sums of between-colony interactions are given in Table 2; for two colonies, A and B, this is equal to

$$\frac{\text{[A butts B]} + \text{[B butts A]}}{n}.$$

Experiment 3—Soldier agonistic behavior. To examine the agonistic responses of soldiers to early instar reproductives, soldiers were obtained from large colonies

containing hundreds of aphids of all stages, because incipient colonies contain few soldiers. Depending on the frequency with which females arrive at already occupied plants, the soldier source colonies may or may not have been pure clones. From each of 27 colonies, a group of soldiers (5.7 ± 3.6 per colony) was collected and placed on a leaf, fixed upside down in a petri dish in the laboratory. The soldiers walked actively on the leaf surface, often with their thickened forelegs raised in an apparently threatening posture. In preliminary tests, other conspecific aphids were placed on the leaf and observed as the soldiers encountered them. In this context, soldiers failed to exhibit any detectable behavioral response to over 50 conspecifics, including soldiers and non-soldiers from their own and other colonies. However, soldiers did respond to early instars held before them in forceps, clasping the aphids with their forelegs and jabbing with their frontal horns. Each soldier was presented with two early instar reproductives held in forceps, one from its own colony and the other from a different site. Responses were recorded blindly; i.e., the origin of the soldiers was unknown to the observer. The order of presentation of the two early instars was alternated, and the forceps were washed between presentations.

RESULTS

Experiment 1—Spatial segregation. Many of the marked aphids disappeared overnight, but sufficient numbers for analysis were recorded in 18 of the 28 replicates, for which the mean number of individuals recovered per colony was 14.0 ± 8.5. To compensate for low recovery rates in some colonies, we combined the 1-1 and 2-2 cells of the contingency table to form one "same-colony" cell, treating the 1-2 cell as the "different-colony" cell for the χ^2 test. In 4 replicates, all individuals from one or both colonies were gone, while in another 6, the numbers remaining generated expected values of less than 5.

The numbers of nearest neighbor pairs composed of aphids from the same and different colonies did not differ significantly in any replicate (Table 1). Though small sample sizes may have weakened the power of some significance tests, neither was there an overall, insignificant trend across replicates. In 10 replicates, the expected number of same-colony nearest neighbors was less than observed; in 7 expected values exceeded observed, with 1 tie ($P = 0.63$, sign test). Members of incipient colonies containing an adult (indicated with an asterisk in Table 1), with a greater certainty of clonal composition, exhibited no greater tendency to aggregate than members of colonies without an adult. Thus there was no evidence of kin-biased spatial segregation.

Experiment 2—Butting behavior. Observation of butting between and within incipient colonies yielded no evidence of agonistic discrimination (Table 2). In 9 out of 10 colony pairs, including 7 in which one or both colonies contained an adult, there was no significant difference between the numbers of butts directed toward colonymates and non-colonymates. Again the negative result could not be explained by sample limitations. With the expected number of between-colony interactions exceeding observed in 4 replicates, observed values exceeded expected in 4, and 2 ties, there was no insignificant trend ($P = 1.0$, sign test). The rate of butting tended to increase in all colonies in the second hour of observation, as the leaves dried out and increasing numbers of aphids sought better feeding sites. Separate analyses of

data from the first and second hours for each colony revealed no differences in bias, however.

In replicate 1, butting was observed significantly less than expected between colonies; that is, putative kin were butted preferentially. Since any individual test in a sufficiently large statistical table may yield significance by chance, the table-wide probability of significant outcomes should also be calculated (Rice, 1989). The table-wide probability of this result is less than 0.05. The first pair of colonies was observed for 3 hours, while all other pairs were observed for 1 or 2, but the result is significant even when only the first 2 hours of data on replicate 1, totalling 49 interactions, are counted: 15 between-colony butts observed, 24 expected, $P < 0.01$, χ^2. However, counting only the first 2 hours, the table-wide probability of a chance result exceeds 0.05.

Of the total of 1,017 butting interactions recorded, 92% were between early instars. However, though few in number, the adults and soldiers also occasionally participated, initiating 3% of butts and receiving 5%. The results of the above analysis were not altered by excluding interactions involving adults and soldiers, while this subset of data was too small to test separately for kinship bias. It is worth emphasizing, though, that early instars did sometimes butt their own putative clonemothers ($N = 15$ occurrences in the entire data set), as well as adults from other colonies ($N = 20$); adults butted their own putative offspring ($N = 9$, vs. 5 for non-colonymates); and sterile soldiers butted their own putative fertile clonemates ($N = 7$, vs. 10 for non-colonymates). Soldiers used their heads when butting, in the same manner as the fertile morphs, never using their forelegs to clasp opponents as observed in experiment 3. Contrary to hypothesis (1a), the soldiers made no attempt to exclude foreigners by butting, clasping or otherwise attacking as they moved among members of both colonies. They ignored other soldiers entirely, though a soldier once butted a foreign adult.

Following many, but not all, successful displacements the aggressor began to feed at the vacated site. However, most aggressors did not succeed in displacing their victims. Of the 94 (9%) successful displacements, 48 were of colonymates, 46 of non-colonymates, while 524 failed butts were directed toward colonymates, 399 toward non-colonymates ($P > 0.05$, χ^2). As Aoki and Kurosu (1985) reported, larger individuals are more successful at displacing smaller ones. Thus adults displaced early instars in 8 of 14 attempts, while an early instar succeeded in moving an adult only once out of 35 attempts. The poor success rate of butting was not the result of elaborate defense behavior. The recipients of butting often rotated or flattened their bodies, but infrequently raised their abdomens in the "headstands" that Aoki and Kurosu (1985) observed in *Astegopteryx bambucifoliae*. On occasion the aggressor and opponent locked horns and rotated around the latter. Very rarely the recipient, its mouthparts still inserted in the plant, butted the aggressor in return. No injuries were observed in any of these interactions.

Experiment 3—Soldier agonistic behavior. When presented with early instar reproductives from their own and other colonies, held in forceps, the sample of 156 soldiers failed to attack aliens significantly more than colonymates. Ninety-nine soldiers aggressively clasped both of the offered early instars between their thickened forelegs, and 22 ignored both. Of the 35 soldiers which responded asymmetrically, 18 clasped the aphid from an alien colony but not the one from their own, and 17

clasped the one from their own colony but not the alien ($P > 0.05$, binomial test). Detection of the early instars was not based on touch or contact chemoreception, since the soldiers raised their forelimbs and began to make clasping motions before contact. They may react to visual stimuli and/or to alarm pheromones contained in the dark brown fluid which these aphids release from their siphunculi when held. Defensive first instars of a monomorphic cerataphidine, *Ceratovacuna lanigera* are known to exhibit an aggressive response to alarm pheromones (Arakaki, 1989).

<div align="center">DISCUSSION</div>

Absence of kin discrimination

Kin recognition could enable soldier or reproductive aphids to expel or avoid unrelated conspecifics, preserving colony clonality (hypothesis 1a); or it could enable soldiers to defend their clonemates discriminatingly within genetically diverse colonies that are compartmentalized into pure clone subgroups (hypothesis 1b). Our results appear to exclude both these hypotheses for *Ceratovacuna japonica*. Soldiers did not discriminate against foreign early instar reproductives which, dispersed by wind from other colonies, are the most probable intruders. Members of different clones showed no tendency to segregate spatially, or to butt non-kin preferentially in competition for feeding sites, which would promote the spatial separation of genotypes. In experiments on three other aphid species, *Pemphigus spyrothecae* (Foster, 1990), *Ceratoglyphina styraciola* (Aoki et al., 1991; Aoki and Kurosu, 1992) and *Pseudoregma bambucicola* (Sakata et al., 1991), soldiers also failed to direct agonistic behavior preferentially against non-colonymates. Kin-biased spatial segregation and butting have not been tested in other soldier-producing species.

Studies of kin recognition must always consider the essential caveat that failure to discriminate cannot prove failure to recognize (Waldman et al., 1988). Any experimental assay of recognition capabilities (except perhaps an electrophysiological one) requires measuring an observable behavioral response, but behavioral responses are often context-dependent. Aphids may spatially segregate by kinship under normal circumstances, but not when suddenly transferred to a new leaf; soldiers may normally exclude non-kin, but attack anything thrust at them with forceps. (Note, however, that this admittedly crude assay was necessitated by the soldiers' failure to respond at all in less contrived encounters.) However, we can conclude that there is no evidence of a simple behavioral mechanism insuring the genetic integrity of the colony, or of spatial subgroups within the colony.

The fact that the sexual phase of the life cycle has been largely suppressed in *C. japonica* might make kin recognition impracticable for this species. Depending on the rates of mutation, sexual reproduction and long-distance dispersal, entire local populations could be effectively pure clones. In that case, there would be no differential inclusive fitness advantage for discriminatory behavior, and insufficient phenotypic variation to permit recognition. (This situation would not be unique to *C. japonica*; some other soldier-producing aphids are also known to produce sexual generations infrequently [Aoki, pers. comm.].) Phenotypic cues acquired from the environment might still vary between colonies as in some, perhaps all, species of social Hymenoptera (Carlin, 1989). Acquired cues presumably would not suffice for

within-colony discrimination, however, given the common chemical environment of cohabiting clones.

We assume that all or most of the incipient colonies used in experiments 1 and 2 were pure clones, and that the absence of significant bias was the result of random interaction. Since the genetic diversity of colonies is undetermined, we cannot exclude the alternative possibility that one incipient colony might contain multiple genotypes, yielding the same results if individuals avoided or butted both unrelated members of their own colony and foreigners, and aggregated with or refrained from butting genuine clonemates. It seems highly unlikely, however, that every one of the newly-founded colonies already contained the offspring of multiple females. Rather, we would expect some colonies (those which were genetically mixed) to exhibit no discrimination, while others (still pure clones) would display quite strong discrimination. Instead, the lack of significant results was highly consistent across replicates. The sole significantly non-random result, in replicate 1 of the butting experiment, cannot be explained by such within-colony nepotism, since kin were butted more often than non-kin.

Butting behavior

In the absence of evidence for discriminatory butting between clones, other explanations for this behavior must be considered. Ordinarily butting is a response to desiccation of the leaves on which the aphids feed, and occurs in overpopulated, aging colonies rather than in tiny, incipient ones. Some individuals give up feeding at unprofitable sites and butt others that are still feeding, in an apparent attempt to displace them; if successful, the aggressor often begins to feed at the vacated site. If colonies become genetically diverse as additional females arrive on occupied plants, such that a colony that has attained sufficient size to damage its host is so heterogeneous that any neighbor is unlikely to be a clonemate, then selection should favor indiscriminate aggression. Reeve (1989) showed that universal intolerance can be selected for even when groups are usually composed of close kin, if the fitness advantage of discriminating against the occasional non-relative is sufficiently higher than the cost of incorrectly rejecting kin. In *C. japonica,* butting of clonemates may represent false-negative errors resulting from a highly restrictive acceptance threshold.

Indiscriminate butting does not necessarily require that colonies are genetically diverse, however. Alternatively, immigration may be negligible, such that large colonies on failing host plants are still pure or nearly pure clones. Inclusive fitness theory does allow competition among close relatives, even clonemates, if on balance the benefit to the genotype exceeds the cost (Hamilton, 1964). This is clearly the case when already-reproducing adult aphids displace early instars, which are only potential reproducers. Generally, early instars should value their 100% related, now reproductive mother much more highly than themselves, but hungry young aphids might attempt to displace their sated mother, as well as well-fed clonemates. More problematic is our observation that sterile soldiers sometimes butt their own fertile clonemates. (Soldiers butting reproductives have also been reported in *Pseudoregma alexanderi* [Aoki et al., 1981].) Still, the soldier's life does have a protective value for its clonemates, and a sated reproductive might occasionally give way to a starving soldier, depending on the cost-benefit ratio.

As a further alternative hypothesis, butting might represent cooperative communication, rather than competition, among members of a pure clone. Desiccation of the host plant represents an emergency condition, to which the colony eventually responds by dispersal (Pierce, Berry and Carlin, in prep.). Butting could constitute a means of spreading information about the imminent decline of the host, facilitating the successful exodus of clonemates. Such information exchange would optimize the allocation of resources: smaller individuals are most easily displaced, and these have the greatest chance of success in wind dispersal. Large adults would do better to monopolize the dwindling food supply and produce a final crop of dispersing first instars. The low probability of successful displacement and the persistence of some unsuccessful "aggressors" may suggest a competitive interaction resisted by the victim, rather than ritualized communication (Aoki, 1987). Nevertheless, the energetic cost of butting could be sufficiently small to be consistent with this scenario.

Evolutionary hypotheses

Our finding that *C. japonica* aphids exhibit no evidence of kin discrimination behavior, in several important contexts in which such behavior is expected, appears to exclude hypotheses (1a) and (1b). Given that the existence of intracolonial butting cannot itself be considered evidence of genetic diversity, the other two possibilities summarized by Ito (1989) remain open: the soldier morph could be maintained by a dispersal pattern tending to preserve clonal integrity (hypothesis 2), or by high predation pressure on mixed colonies (hypothesis 3). Though soldier aphids are often cited as a paradigmatic product of inclusive fitness (e.g., Trivers, 1985), it is worth noting that hypothesis (3) might well operate by group, not kin, selection. Soldier production would be evolutionarily stable in multi-clone colonies only if each "cheater" female suffered an immediate and direct fitness cost from failing to contribute to the collective defense. Females that joined colonies but did not produce soldiers would prosper temporarily, but ultimately suffer the eradication of their group. This is a straightforward structured-deme model of group selection (Wilson, 1980) in which the groups are composed of multiple clones, rather than unrelated individuals. The fact that groups happen to consist of multiple clones should make no difference, however. The great efficiency with which predatory insects can devastate entire aphid colonies (Pierce, Berry, and Carlin in prep.) could provide the requisite high rate of group extinction as well as the need for defense. Since cheaters are expected to increase locally, this hypothesis yields the experimentally testable prediction that soldier production will soon decline or cease in an aphid population that is shielded from predators.

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